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A stochastic interspecific competition model to predict the behaviour of *Listeria monocytogenes* in the fermentation process of a traditional Sicilian salami

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Abstract

The present paper discusses the use of modified Lotka-Volterra equations in order to stochastically simulate the behaviour of *Listeria monocytogenes* and Lactic Acid Bacteria (LAB) during the fermentation period (168 h) of a typical Sicilian salami. For this purpose, the differential equation system is set considering T, pH and aw as stochastic variables. Each of them is governed by dynamics that involve a deterministic linear decrease as a function of the time t and an "additive noise" term which instantaneously mimics the fluctuations of T, pH and aw. The choice of a suitable parameter accounting for the interaction of LAB on *L. monocytogenes* as well as the introduction of appropriate noise levels allows to match the observed data, both for the mean growth curves and for the probability distribution of *L. monocytogenes* concentration at 168 h.

Keywords: Predictive microbiology; Interspecific competition model; Stochastic approach; Environmental noise; Listeria monocytogenes; Lactic Acid Bacteria.

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I. INTRODUCTION

Predictive microbiology aims to develop accurate and versatile mathematical models able to describe microbial evolution in food products as a function of environmental conditions. According to Whiting and Buchanan [1], predictive models are classified as primary, secondary and tertiary. Primary models describe microbial evolution as a function of time. Secondary models relate parameters which appear in primary modelling to environmental conditions such as temperature (T), pH, water activity (aw), etc. Tertiary models combine primary and secondary models (e.g., the Pathogen Modelling Program, designed by the USDA or the Growth Predictor developed by IFR) including, in more extended versions, the possibility to import a T history in order to predict remaining shelf life with regard to Specific Spoilage Organisms, as in the Seafood Spoilage Predictor [2]. Modelling the microbial evolution as a function of fluctuating environmental conditions (dynamic models) is very important for practical predictive applications, especially when the technology of some products, such as ripened meats and cheeses, is based on a continuous modification of T, pH, relative humidity (RH), etc. However, for these kinds of products, dynamic models [3, 4, 5] can lead to overestimate the real bacterial growth if they do not take into account the inhibition by the competitive natural microflora. The bacterial competition within the natural microflora is a complex issue in modelling microbial evolution and it has been studied by several authors [6, 7, 8, 9]. An interesting approach to describe bacterial competition is based on the generalized Lotka-Volterra model [10, 11]. This provides basic equations for the population growth of two interacting species. A prototype model structure for mixed microbial populations in food products was proposed by Dens et al. [12]. This consists of a system of four differential equations and combines the advantages of the Lotka-Volterra model for two competing species with those of Baranyi & Roberts' model [13] as a classical predictive growth model. Powell et al. [14] used the above mentioned system of equations in order to interpret some empirical results for *Escherichia coli* O157:H7 in ground beef, and they showed that the seemingly incongruous data was consistent with the interspecific competition model. Furthermore, the authors considered the effect of varying the growth rates stochastically, introducing an uniform random distribution for ?max of both species into the model; in this way, the incorporation of microbial community dynamics into the food safety risk assessment process was explored. The construction of probabilistic predictive

models, based on stochastic dynamics, is very important for the relation between predictive microbiology and quantitative risk assessment. An extensive review on predictive microbiology [15] indicated that the use of stochastic models produces remarkable effects, since it allows to move away from the worst-case scenario. In this regard, Nauta [16], discussing the separation of uncertainty and variability in quantitative microbial risk assessment models, proposed the introduction of some stochastic terms into a system consisting of primary and secondary growth models [17]. A very different approach to stochastically model bacterial growth and inactivation is based on considering the microbial population behaviour as the mean of the behaviour of many cells [18, 19, 20]. In this case, measuring the single cell growth or survival parameters, and repeating these measurements for a wide number of cells, it would be possible to express Lag-time and μ_{max} in terms of probability distributions. This theoretical approach, further discussed in Kutalik et al. [21], was used by Métris et al. [22] for *E. coli*, Francois et al. [23] and Francois et al. [24] for L. monocytogenes. In a recent paper Ponciano et al. [25] presented a new application of a stochastic ecological model based on the stochastic version of the well-known Verhulst logistic differential equation. By using an extensive experimental data set and requiring the specification of a likelihood function, this stochastic model allows to analyse the influence both of deterministic and random variations of the environmental conditions on microbial growth dynamics. In light of the above trends in predictive microbiology, the development of new models should satisfy three important requirements: i) the application of a dynamical approach, namely based on the use of differential equations; ii) the presence of interactions among food microbial communities; iii) the presence of stochastic terms in equations in order to take into account the influence of random variations of the environmental conditions. An interesting answer to the aforementioned requirements could be provided by incorporating stochastic growth rates into an interspecific competition model described by the Lotka-Volterra equations. The dynamics of the species can be affected by random fluctuations directly, through a term of multiplicative noise in the species equations, or indirectly, through a term of additive noise which mimics the fluctuations of environmental conditions [26, 27]. The aim of this work is to propose a stochastic approach for predictive microbiology. More specifically, we add noise terms in the time evolution of three basic parameters T, pH, aw, in an attempt to obtain a stochastic model for the bacterial growth dynamics, starting from the above mentioned generalized Lotka-Volterra equations. This approach was used in order to stochastically simulate the

behaviour of L. monocytogenes and Lactic Acid Bacteria (LAB) during the fermentation step of a typical Sicilian salami; the model was validated by taking into account the data of a previous challenge test for L. monocytogenes [28]. We have chosen to consider only the fermentation step since, as well known, the fluctuations of T, pH and aw are wider in this phase than in the dry-curing, where T especially remains constant. Furthermore, the aim of this work is to evaluate L. monocytogenes behaviour under conditions of potential growth and in the presence of LAB competitive activity while, during the dry-curing in strict sense, the values of T, pH and aw fall below the L. monocytogenes "growth region" [29].

II. MATERIALS AND METHODS

A. Model

The stochastic interspecific competition model is structured in order to simulate the behaviour of L. monocytogenes and Lactic Acid Bacteria (LAB) during the fermentation step (7 days) of a traditional Sicilian salami. According to the data of a previous work [28], this is done considering T, pH, and aw linearly decreasing respectively from 20C to 12C, from 5.8 to 5.6, [28]and from 0.972 to 0.946, during 168 h. The system of four differential equations proposed by Dens et al. [12] and Powell et al. [14],

$$\frac{dN_{Lmo}}{dt} = \mu_{max\,Lmo}\, N_{Lmo} \frac{Q_{Lmo}}{Q_{Lmo}+1}\left(1 - \frac{N_{Lmo} + \beta_{Lmo/LAB}\, N_{LAB}}{N_{max\,Lmo}}\right) \quad (1)$$

$$\frac{dQ_{Lmo}}{dt} = \mu_{max\,Lmo} Q_{Lmo} \quad (2)$$

$$\frac{dN_{LAB}}{dt} = \mu_{max\,LAB}\, N_{LAB} \frac{Q_{LAB}}{Q_{LAB}+1}\left(1 - \frac{N_{LAB} + \beta_{LAB/Lmo}\, N_{Lmo}}{N_{max\,LAB}}\right) \quad (3)$$

$$\frac{dQ_{LAB}}{dt} = \mu_{max\,Lmo} Q_{LAB}, \quad (4)$$

was used as basic model. Here NLmo and NLAB are, respectively, the population densities of L. monocytogenes and Lactic Acid Bacteria at time t; $\beta_{max\,Lmo}$ and $\beta_{max\,LAB}$ are the maximum specific growth of both species and $N_{max\,Lmo}$ and $N_{max\,LAB}$ are the theoretically maximum population densities of both species under monospecific growth conditions. The coefficients $\beta_{Lmo/LAB}$ and $\beta_{LAB/Lmo}$ are, respectively, the interspecific competition parameters of LAB on L. monocytogenes and vice-versa. Q_{Lmo} and Q_{LAB} represent, respectively, the physiological state of the two species. Furthermore, according to Baranyi and Roberts [13],

we obtain the Lag-time

$$\lambda(t) = \frac{-\ln \alpha(t)}{\mu_{max}(t)} \tag{5}$$

with $\alpha(t)$ given by

$$\alpha_{Lmo}(t) = \frac{Q_{Lmo}(t)}{1 + Q_{Lmo}(t)} \tag{6}$$

$$\alpha_{LAB}(t) = \frac{Q_{LAB}(t)}{1 + Q_{LAB}(t)}. \tag{7}$$

The below second order model Eq. (8) (so-called Ratkowsky or square root type model), developed by Tom Ross at the University of Tasmania (UTAS model) and reported by Giménez and Dalgaard [7], was incorporated into Eq. (1) while T , pH and aw were time-dependent.

$$\begin{aligned}\sqrt{\mu_{max\,Lmo}} &= 0.14776 \cdot (T - 0.88) \cdot (1 - e^{0.536\,(T-41.4)}) \cdot \sqrt{Aw - 0.923} \cdot \sqrt{1 - 10^{4.97-pH}} \\ &\cdot \sqrt{1 - \frac{LAC}{3.79(1 + 10^{pH-3.86})}} \cdot (350 - NIT)/350,\end{aligned} \tag{8}$$

where the values 0.88, 41.4, 0.923, 4.97 and 350 represent respectively T_{min} ($^{\circ}C$), $Tmax$ ($^{\circ}C$), aw_{min}, pH_{min} and NIT_{max} (nitrite concentration in ppm). LAC is the lactic acid concentration ($g\,l^{-1}$) and was obtained by using the following system of differential equations, proposed by Leroy and De Vuyst [30] and Leroy et al. [31]

$$\frac{dLAC}{dt} = -YLAC_S \frac{dS}{dt} \tag{9}$$

$$\frac{dS}{dt} = \frac{-1}{YLAB_S \frac{dN_{LAB}}{dt} - m_S LAB}. \tag{10}$$

Here YLACS is the yield coefficient for the production of lactic acid from the fermentable sugar S ($g\,l^{-1}$), YLABS and mSLAB are two coefficients which express the depletion of fermentable sugar S as a function of the instantaneous modifications of Lactic Acid Bacteria concentration (dN_{LAB}/dt; see equations 1c and 1d). In order to solve the equation 1c we used the below second order model proposed by Wijtzes et al. [32]

$$\mu_{max\,LAB} = -0.00234 \cdot (aw - 0.928) \cdot (pH - 4.24) \cdot (pH - 9.53) \cdot (T - 3.63)^2, \tag{11}$$

where the values 0.928, 4.24, 9.53 and 3.63 represent respectively aw_{min}, pH_{min}, pH_{max} and T_{min}. According to Leroy and De Vuyst [30], the term YLACS (Eq. (9)) was set to 1, while

the following models were used in Eq. (13)

$$YLAB = 3.3 \cdot (6.10^{-4}\,T^2 - 0.044\,T + 1.03) \cdot (-0.13\,pH^2 + 1.48\,pH - 3.88) + 0.035 \quad (12)$$

$$m_S LAB = 0.3 \cdot (-0.1583\,pH^2 + 1.98\,pH - 5.59) \cdot YLAB_S^{-1} - 0.23. \quad (13)$$

For the initial value of Q_0, the procedure of Baranyi et al. [3] was followed: a geometric mean value for the physiological state parameter $\alpha(t)$ was estimated from several growth curves of both bacterial populations, obtained from ComBase (http://combase.arserrc.gov; IFR, Norwich, UK). The same procedure was used in order to set the Nmax values, while the initial bacterial values were those of a previous study [28] which were used to validate the present model (see the related section). Finally, the system was solved, by numerical simulations, to obtain predictions for the bacterial concentration during time-dependent T / pH / aw profiles.

B. Noise and stochastic dynamics

In the above section we have introduced a model of two interacting species based on generalized Lotka-Volterra equations [10, 11, 27], where the growth rates of the two species depend on T, pH and aw. We assume that these three parameters are driven by deterministic forces, which model the external conditions imposed, for example, by the production standards used in the food industry. However, real ecosystems interact with a noisy nonstationary environment, so that parameters such as T, pH and aw, are also affected by random fluctuations. To describe this continuous and noisy behaviour of T, pH and aw, we consider the following stochastic differential equations [33]

$$\frac{dT(t)}{dt} = k_T\,t + \xi_T(t) \quad (14)$$

$$\frac{dpH(t)}{dt} = k_{pH}\,t + \xi_{pH}(t) \quad (15)$$

$$\frac{daw(t)}{dt} = k_{aw}\,t + \xi_{aw}, \quad (16)$$

where the deterministic terms depend linearly on the time t and the random terms $\xi_T(t)$, $\xi_{pH}(t)$, $\xi_{aw}(t)$ mimic the fluctuations that affect T, pH, and aw, considering their interaction with the environment. The coefficients k_T, k_{pH}, k_{aw} are the rates of T, pH and aw, respectively. $\xi_T(t)$, $\xi_{pH}(t)$, $\xi_{aw}(t)$ are statistically independent Gaussian white noises with

zero mean and correlation functions $< \xi_T(t)\xi_T(t') >= \sigma_T\, \delta_T(t-t')$, $< \xi_{pH}(t)\xi_{pH}(t') >= \sigma_{pH}\, \delta_{pH}(t-t')$, $< \xi_{aw}(t)\xi_{aw}(t') >= \sigma_{aw}\, \delta_{aw}(t-t')$. Here σ_T, σ_{pH}, σaw are the standard deviations of the three Gaussian distributions, and they are the intensities of the noise sources which affect T, pH, and aw.

C. Scenarios

Eqs. (1)-(4) were solved numerically, obtaining the time series of the species concentrations for different scenarios, that correspond to different values of the noise intensities and interaction parameters. Table I summarizes the Lotka-Volterra competition model parameters

			noise intensity		
scenario	$\beta_{Lmo/LAB}$	$\beta_{LAB/Lmo}$	T	pH	aw
1	0.0	0.0	0.0	0.0	0.0
2	0.656	0.0	0.0	0.0	0.0
3	0.656	0.0	10^{-2}	$5*10^{-4}$	10^{-5}
4	0.656	0.0	$2*10^{-1}$	10^{-4}	$1.5*10^{-4}$
5	0.656	0.0	$5*10^{-1}$	$5*10^{-4}$	10^{-5}

TABLE I: Competition model parameters used for each scenario. Scenario 1 assumes the absence of interaction between species and a zero noise intensity. The "zero intensity" of noise is maintained in the scenario 2, but the interaction term of LAB on *L. monocytogenes* is introduced. In scenario 3, a low intensity of noise is introduced for T, pH and aw so that the fluctuations of the three environmental variables affect weakly the growth parameter $\mu_{max\,Lmo}$. In scenarios 4 and 5 the noise intensity progressively increases whereas the interaction terms remain constant.

for the scenarios considered in this paper. For every scenario we solved Eqs. (1)-(4), (5), (9)-(13) by numerical integration, performing 1000 realizations. The initial conditions for the two specie concentrations were chosen, in each realization, by setting a Gaussian distribution with mean and standard deviation equal to those experimentally observed [28]. In particular, as a mean value and standard deviation we used, respectively, Log 2.492 cfu/g and 0.170 for *L. monocytogenes*, and Log 6.825 cfu/g and 0.688 for LAB. Scenario 1, used as a control scenario, assumes the absence of interaction between species and a zero noise intensity. In

this case the system works like the logistic model of Baranyi et al. [13] in the presence of decreasing environmental conditions (T from 20 $^{\circ}C$ to 12 $^{\circ}C$, pH from 5.8 to 5.6, and aw from 0.972 to 0.946). The "zero intensity" of noise is maintained in scenario 2, but introducing the interaction term of LAB on *L. monocytogenes* ($\beta_{Lmo/LAB} = 0.656$). In scenario 3, a low intensity of noise is introduced for T ($T = 10^{-2}$), pH ($pH = 5 \cdot 10^{-4}$) and aw ($aw = 10^{-5}$) so that the fluctuations of the three environmental variables (T, pH, aw) weakly affect the growth parameter $\mu_{max\,Lmo}$. In scenarios 4 and 5 the noise intensity progressively increases whereas the interaction terms remain constant. Note that, in scenarios 2, 3, 4, 5 (see table 1), the absence of any competitive effects of *L. monocytogenes* on LAB ($\beta_{LAB/Lmo} = 0$), implies $\beta_{Lmo/LAB} < N_{max\,Lmo}/N_{max\,LAB}$, as a coexistence condition. The value of $\beta_{Lmo/LAB}$ (see table 1) and those fixed for the maximum population densities, $N_{max\,Lmo} = 7.5$ and $N_{max\,LAB} = 9.3$, satisfy the previous inequality, so that a coexistence regime for *L. monocytogenes* and LAB is established [12, 26, 27]. This agrees with the empirical data on the L. monocytogenes behaviour during the seasoning of salami [28, 34, 35, 36, 37, 38]. The nitrite concentration was set constantly at 90 ppm, according to the data of a previous work [28].

D. Validation of the model

In order to validate the deterministic and stochastic predicted results, we considered the experimental data obtained in a previous study [28] in a challenge test for L. monocytogenes in a typical Sicilian salami. In particular, the data referred to 63 samples of S. Angelo Salami PGI (Protected Geographical Indication; EU regulation 510/06) inoculated, after the stuffing into a natural pork casing, according to the indication of Scott et al. [39], with a suspension (5 ml; Log $4,320$ cfu/ml) of a strain of L. monocytogenes previously isolated in the same kind of salami. The experimental contamination was performed by inoculating the suspension of L. monocytogenes into 20 different sites of each sample. Products, with an average weight 500 ± 10g and diameter of 50-60 mm, were coarse-grained and contained 3% of NaCl, 100 ppm of nitrate and 90 ppm of nitrite, according to the related product specification. Before the stuffing, a commercial mixture of Lactobacillus plantarum and Staphylococcus xylosus, for one group of samples, and a mixture of Pediococcus acidilactici, for a second group, were used. In the present study, however, the two groups were considered together, since, as previously reported [28], the evolution of LAB and L. monocytogenes as

well as pH, aw and weight loss were very similar. Nine products for each group were analysed in triplicates at 0, 48, 120 and 168 hours after stuffing, during the fermentation step (7 days), with regard to the determination of L. monocytogenes count, Lactic Acid Bacteria, pH and aw. The environmental temperature and RH were monitored by a data logger (FT-102 Econorma, Treviso - Italy), in order to check the fermentation programme characterized by a gradual decrease of temperature from 20 $^{\circ}C$ to 12 $^{\circ}C$ and a gradual increase of RH from 63% to 70%, within the considered time interval (168 h). In this study we performed experiments using a single inoculum level (Log 2.492 cfu/g). This condition does not affect the validity of our analysis since we expect that the inoculation level plays a marginal role in the bacterial dynamics when the system is far from strongly stressed conditions and the initial concentration is greater than 1 Log cfu/g [40]. Therefore, we compared experimental and theoretical results by using a single inoculation level over 54 trials and one profile of T, pH and aw to obtain a statistical distribution for the concentration at different times. In particular, the *L. monocytogenes* predicted mean growth curve was compared to the observed one and the differences were statistically analysed through the Root Mean Squared Error (RMSE). Furthermore, the chi-squared test was used in order to evaluate the differences among the probability distributions of the observed and predicted *L. monocytogenes* concentrations at 168 hours. It is worth noting that the above study, based on a single experiment with 54 trials, was not considered in view of a complete validation of the model, since the aim of the work was to show the role of environmental noise on bacterial dynamics. For this purpose, we took into account a data set where the variability is strongly reduced, in order to highlight the effects of the random fluctuations of the environmental parameters, through a comparison between observed data and theoretical results. However, a validation of our model, in view of a general application to the dynamics of two bacterial competing species, needs wider investigation and it will be the subject of a forthcoming paper.

III. RESULTS AND DISCUSSION

A. Deterministic behaviour (scenarios 1-2)

The observed mean behaviour of *L. monocytogenes* (Fig. 1) is characterized by a slight increase and a subsequent decrease until a final (168 hours) concentration given by Log 2.370 ± 0.214 cfu/g. This is very close to the initial (0 hours) value, Log 2.492 ± 0.170 cfu/g. The prediction carried out by using the parameters of Scenario 1 (interaction terms and noise intensity set to 0) provides a behaviour which is very different from the experimental data. In Fig. 1 the black line with little dashes represents the time behaviour of the

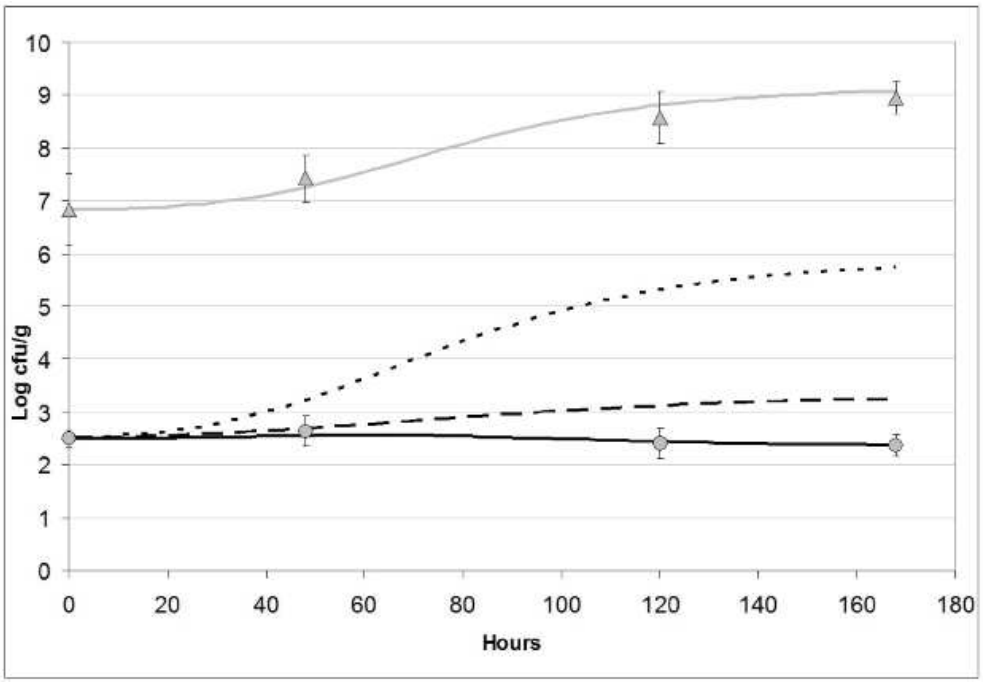

FIG. 1: Observed mean bacterial behaviour (*L. monocytogenes*: circles; LAB: triangles). Error bars indicate ± 1 standard deviation. Black line with little dashes and black solid line represent the predicted values of *L. monocytogenes* concentration for scenarios 1 and 2, respectively. Gray line indicates the predicted LAB growth for both scenarios. The three curves are obtained with nitrite concentration set at 90 ppm. The black line with large dashes shows the prediction of L. monocytogenes concentration for scenario 1 with nitrite concentration at 150 ppm.

L. monocytogenes concentration for scenario 1. Here, the maximum predicted value of the *L. monocytogenes* concentration is lower (Log 5.724 cfu/g) than the theoretical Maximum

Population Density $N_{max\,Lmo}$ (Log 7.5 cfu/g), and closer to the observed value. However, the predicted behaviour of *L. monocytogenes* for scenario 1 is very different (RMSE = 2.2386) from the observed data (circles in Fig. 1). This indicates that the effect of environmental hurdles, such as the lactic acid concentration modelled according to equations 5a-b, cannot explain the complexity of the considered microbiological system. A better prediction is obtained for scenario 1 by setting the nitrite concentration at 150 ppm (instead of 90 ppm used in this work, according to the previous work [28]). However, also in this case the predicted growth (black line with large dashes in Fig. 1) does not agree with the observed behaviour (RMSE = 0.5634). In scenario 2, according to Powell et al. [14], the interspecific interaction is considered. We find that a suitable value of $\beta_{Lmo/LAB}$ exists for which the mean *L. monocytogenes* behaviour (black solid line in Fig. 1) is very close to the observed one (RMSE = 0.0449). This result shows the fundamental role played by the interspecific competition in view of investigating the bacterial dynamics of *L. monocytogenes* and LAB communities. The Lotka-Volterra approach is able to simulate the competition between two populations, describing different situations such as the mutual interaction, the reduction of only one population ("low or no growth") or the decline. These effects are related to the bacterial concentration which, however, depends only on environmental conditions since the interaction parameters, $\beta_{Lmo/LAB}$, do not affect the maximum growth rate. The approach based on the "Jameson effect" hypothesis [40] gives a phenomenological description of *L. monocytogenes* behaviour in real food, that is, in the presence of other bacterial species, i.e. competitors. In fact, according to the "Jameson effect" hypothesis, due to the bacterial interaction, a different value of Nmax is measured, without providing a "dynamical" explanation for this new value. Conversely, previously measuring the N_{max} value for *L. monocytogenes* in monoculture allows us to acquire knowledge of the "free" (in the absence of bacterial competition) behaviour of *L. monocytogenes*, and to obtain the effect of the competition by introducing an interaction term. The comparison between experimental data, obtained in competition regime, and theoretical results, calculated by using, in the competition model, the growth parameters previously obtained from monoculture experiments, indicates what the effect of the bacterial interaction is. At the same time this allows to determine the values of the interaction parameters, $\beta_{Lmo/LAB}$ and $\beta_{LAB/Lmo}$, for which the theoretical distributions are in good agreement with the experimental ones. Note that, in scenario 1, the model works as a conventional predictive system (e.g. Baranyi and Roberts model) where the bac-

terial behaviour is only governed by the three parameters Q, μ_{max}, N_{max}. In particular, Q and μ_{max} are related to environmental characteristics through a secondary predictive model which derives from a monoculture set of experimental data, while the limiting term N_{max} is a static parameter. In scenario 2, the conventional predictive approach describes the gradual transition of the system to the Lotka-Volterra dynamics. In fact, according to Eqs. (1,2,3,4), the interaction terms, which depend on the bacterial concentrations, begin to express the competitive effect of LAB on *L. monocytogenes* when the system leaves the Lag-phase. This approach differs from that of Leroy et al. [41] which studied the competitive interaction of Lactobacillus sakei on *L. monocytogenes*. According to their previous studies [30, 31] they modelled the production and the activity of the bacteriocin by using mono- and co-culture in-vitro conditions, showing a non-constant activity of bacteriocins. In view of this aspect and taking into account the real complexity of the considered food system, characterized by the heterogeneity of LAB and presence of other interaction mechanisms different from the bacteriocin production [42], we have chosen to describe the interaction mechanism of LAB on *L. monocytogenes* by using a single term ($\beta_{Lmo/LAB}$).

B. Stochastic behaviour (scenarios 3-5)

In Fig. 2 we show the probability distribution of *L. monocytogenes* concentration at 168 hours for scenario 2 (panel a), scenario 3 (panel b), scenario 4 (panel c) and scenario 5 (panel d), compared to the observed probability distributions. Table II reports the results for the chi-squared test, RMSE and mean value of the *L. monocytogenes* concentration for all scenarios. As Table 2 and Fig. 2 show, the increase of noise produces a reduction of the mean value of the L. monocytogenes concentration at 168 h. In particular, for scenarios 2 and 3 (0 or low noise intensities) the central part of the predicted probability distribution takes on values significantly larger than the observed one (panels a and b of Fig. 2). For higher levels of noise the values of the theoretical probability distribution are reduced, and the predicted and observed probability distributions are in very good agreement (Fig. 2c). A further increase of the noise intensity causes the predicted probability distribution to become very different from the observed one (Fig. 2d). Moreover, concerning the RMSE values, as shown in Table 2, the noise intensity of scenario 4 allows an enhancement of predictive model performances to be obtained, producing the lowest RMSE value. Conversely,

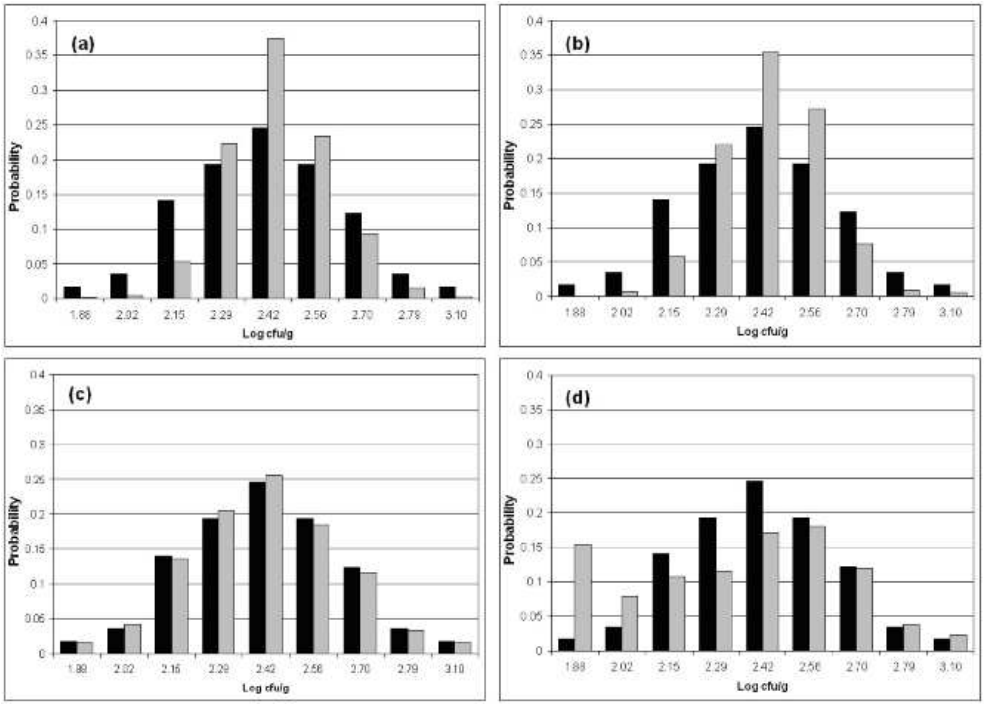

FIG. 2: . Observed (black bars) and predicted (gray bars) probability distributions of *L. monocytogenes* concentration at 168 hours for scenarios 2 (panel a), 3 (panel b), 4 (panel c) and 5 (panel d). The total number of trials in the experimental work is 54. In the theoretical approach we performed 1000 iterations.

	Log cfu/g ± standard deviation				RMSE	Chi-squared *
	0 h	48 h	120 h	168 h		
Observed data	2.492 ± 0.170	2.62 ± 0.284	2.40 ± 0.281	2.370 ± 0.214	-	-
Scenario 1	2.492 ± 0.170	3.215 ± 0.188	5.308 ± 0.161	5.724 ± 0.138	2.2386	-
Scenario 2	2.492 ± 0.170	2.546 ± 0.159	2.439 ± 0.151	2.374 ± 0.136	0.0449	211.56
Scenario 3	2.492 ± 0.170	2.546 ± 0159	2.441 ± 0.151	2.373 ± 0.144	0.0457	218.58
Scenario 4	2.492 ± 0.170	2.547 ± 0.167	2.404 ± 207	2.341 ± 0.216	0.0432	4.40
Scenario 5	2.492 ± 0.170	2.535 ± 0.174	2.313 ± 0.227	2.262 ± 0.319	0.0840	1183.37

TABLE II: . Observed and predicted *L. monocytogenes* mean values at 0, 48, 120 and168 hours, for each scenario. The Root Mean Squared Error (RMSE) indicates the agreement of the mean predicted curves to the observed ones in each scenario. The chi-squared values are referred to the *L. monocytogenes* probability distribution at 168 h for scenarios 2, 3, 4 and 5.

a lower noise intensity (scenario 3), as well as a higher one (scenario 5), produces a reduced fitting of the *L. monocytogenes* mean concentration with the observed data (Table II). Furthermore, in scenarios 2 and 3, the simulation provides a distribution characterized by a small standard deviation and pronounced symmetry around the central value. On the other hand, in scenario 5, the data distribution (with a high standard deviation) shows a marked peak at the minimum value. Therefore, the overall evaluation of these results, obtained from stochastic dynamics, shows that the environmental noise causes a global effect consisting of a reduction of the *L. monocytogenes* mean value concentration. However, at the same time, a suitable level of noise intensity allows to obtain bacterial growth values, whose probability distribution matches the observed one very well. This is the most relevant result of the present study. It is important to stress that we are not interested in measuring the intensity of the noise that affects the experimental data. However, environmental parameters, i.e. T, pH and aw, undergo random fluctuations, always present in "open systems" such as that considered in this work. Here, we intend to show that the observed data distributions, can not be reproduced by the proposed model in the absence of noise (scenario 2). Conversely, a suitable level of noise allows to obtain theoretical results in good agreement with experimental findings. This aspect could play a key role in view of incorporating stochastic microbial predictive models (such as the proposed one) into a risk assessment process, since the introduction of the appropriate level of noise can influence the precision in the expression of the probabilistic "output" related to the concentration of a foodborne disease agent. In our study, for example, the observed percentage of samples with a *L. monocytogenes* concentration at h 168 $\leq$ Log 2.0 cfu/g (regulatory critical limits in EU) was 4.4%, while the predicted percentages in scenarios 3, 4 and 5, were, respectively, 0.4%, 5.2% and 22%. Note that the separation between uncertainty and variability, which is usually fundamental in the application of stochastic models [16, 43], was not introduced in the present study, since the observed data are obtained by a single strain of *L. monocytogenes*. In general, the bacterial cells, obtained from the same strain, should exhibit the same average biological and physiological properties. Therefore, our inoculations, and then the corresponding concentrations, belong to the same statistical distribution. This means that the hyperparameters, i.e. expected value and standard deviation, are the same for the initial concentrations used in the experimental trials [43]. This condition suggests the absence of variability in the initial conditions over the different trials, while the uncertainty, which is connected with the difficulty

to inoculate all salami at precisely the same concentration and the technical uncertainty such as the error of the enumeration method, was expressed by a Gaussian distribution with mean and standard deviation equal to the experimentally observed ones (Log 2.492 ± 0.170 cfu/g for *L. monocytogenes*). However, the variability could be considered by changing the growth rate parameter for each different strain in our Lotka-Volterra stochastic model. Another interesting consideration regards the enhancement of predictive model performances obtained by increasing the noise until the level of scenario 4 (RMSE values, Tab. 2). From a biological point of view, this accounts for the environmental noise, that is, random fluctuations of external variables such as T. The presence of noise influences the growth rate and, indirectly, the interaction between bacterial species. It is important to recall that in many fields where population dynamics is studied, the noise effects on ecological systems are the subject of an intensive investigation [44, 45, 46]. Theoretical analyses and experimental results have showed that noise, in the presence of nonlinear dynamics, is responsible for several counterintuitive phenomena, such as stochastic resonance [47, 48, 49, 50, 51, 52, 53, 54, 55], noise enhanced stability [56, 57, 58, 59, 60], and noise delayed extinction [27, 61], which are not present in purely deterministic regimes. Therefore, noise and its effects have become a well established subject in physics, chemistry, and biology [62]. The contemporary presence of noise and nonlinear interactions in ecological systems is responsible for the appearance of a rich dynamics, which corresponds to the real complexity of natural systems. From a theoretical point of view, this situation can be described by using a model where both the internal nonlinear interactions of the system and the noisy interaction with the environment are taken into account, giving rise to a complex behaviour of the system, which is very sensitive to initial conditions, various deterministic external perturbations and random fluctuations always present in nature. This paper presents a further evolution of the interspecific competition model proposed by Dens et al. [12] and Powell et al. [14], in order to reproduce the complexity of some food systems during their production. In this regard, taking into account the generalized Lotka-Volterra equations, we introduced T, pH and aw as stochastic variables. The dynamics of T, pH, aw, affecting bacterial rate, obey stochastic differential equations that involve both a deterministic term, varying linearly as a function of time, and a random term, that is responsible, at each time t, for fluctuations (noisy behaviour) of T, pH, aw. Initially, we considered the deterministic decrease and increase, respectively, of T and RH in the seasoning rooms and the bacterial metabolic activity (e.g. sugar fer-

mentation). Moreover, the T and RH of seasoning rooms can be also affected by random fluctuations, beside the decrease imposed by production standards. In this way, in principle, each single cell composing a food bacterial population interacts with a different environment at each time t, having a different Lag-time and growth rate. The introduction of stochastic terms, expressed by equations 14, 15, 16, into the Lotka-Volterra equations reproduces the presumable noisy behaviour which affects the Lag-time, the growth rate and therefore the bacterial concentration proportionally to the noise intensity, allowing the transition from a deterministic to a stochastic predictive model. The probabilistic model used in this work has some similarities with that of Francois et al. [23] and Francois et al. [24] for *L. monocytogenes*, but the approach methodology is very different. In fact, the above mentioned authors measured empirically the fluctuations of the species concentrations at constant environmental conditions (individual-based approach), obtaining a statistical distribution by fitting the different data sets. In our case, the data distribution is the consequence of environmental noise, modelled as a white Gaussian-distributed noise, whose SD represents the intensity. Therefore, while the data distribution obtained with the "individual-based approach" accounts for intra-specific differences in adaptation to environmental parameters, our approach mainly considers different cell behaviour as a consequence of environmental heterogeneity. As this study shows, the real behaviour of *L. monocytogenes* in meat products during the fermentation step is mainly affected by bacterial interactions which are, however, dependent on environmental fluctuations too. Moreover, other implications could be explored using, for example, a time correlated noise as theoretically suggested by Spagnolo et al. [26] or introducing a "multiplicative noise" [26, 63, 64], which directly affects specie concentrations. A further development of the stochastic dynamical model proposed in this paper could also consist of using a growth/no-growth term [29, 65, 66]. This implies that fluctuating environmental conditions could cause the growth rate to go below a given threshold (no-growth region), contributing to the generation of a richer dynamics: both growth and no-growth cells of a bacterial population could have a non-vanishing probability to appear at the same time. Therefore, the model, in a probabilistic sense, should be predictive: it could allow to calculate which is the probability that, at a certain time t, the bacterial growth (or no-growth) takes a given value. In conclusion, our approach as well as all its further developments could be useful for the incorporation of the predictive microbiology models

into the quantitative risk assessment process.

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[1] Whiting RC, Buchanan RL (1993) Food Microbiol 10:175-177.

[2] Dalgaard P, Buch P, Silberg S (2002) Int J Food Microbiol 73:343-349.

[3] Baranyi J, Robinson TP, Kaloti A, Mackey BM (1995) Int J Food Microbiol 27:61-75.

[4] Bovill R, Bew J, Cook N, D'Agostino M, Wilkinson N, Baranyi J (2000) Int J Food Microbiol 59:157-165.

[5] Xanthiakos K, Simos D, Angelidis AS, Nychas GJ-E, Koutsoumanis K (2006) J Appl Microbiol 100:1289-1298.

[6] Pin C, Baranyi J (1998) Int J Food Microbiol 41:59-72.

[7] Giménez B, Dalgaard P (2004) J Appl Microbiol 96:96-109.

[8] Vereecken KM, Devlieghere F, Bockstaele A, Debevere J, Van Impe JF (2003) Food Microbiol 20:701-713.

[9] Van Impe JF, Poschet F, Geeraerd AH, Vereecken KM (2005) Int J Food Microbiol 100:97-105.

[10] Lotka A J (1920) Proc Nat Acad Sci USA 6:410-415

[11] Volterra V (1926) Nature 118:558-560

[12] Dens EJ, Vereecken KM, Van Impe JF (1999) J Theor Biol 201:159-170.

[13] Baranyi J, Roberts TA (1994) Int J Food Microbiol 23:277-294.

[14] Powell M, Schlosserb W, Ebel E (2004) Int J Food Microbiol 90:171-179.

[15] McMeekin TA, Olley J, Ratkowsky DA, Ross T (2002) Int J Food Microbiol 73:395- 407.

[16] Nauta M J (2000) Int J Food Microbiol 57:9-18.

[17] Ratkowsky DA, Olley J, McMeekin TA, Ball A (1982) J Bacteriol 149:1-5.

[18] Baranyi J (1998) J Theor Biol 192:403-408.

[19] Baranyi J, Pin C (2001) J Theor Biol 210:327-336.

[20] Swinnen IAM, Bernaerts K, Dens EJJ, Geeraerd AH, Van Impe, JF (2004) Int J Food Microbiol 94:137-159.

[21] Kutalik Z, Razaz M, Baranyi J (2005) J Theor Biol 232:285-299.

[22] Métris A, Le Marc Y, Elfwing A, Ballagi A, Baranyi J (2005) Int J Food Microbiol 100:13-19.

[23] Francois K, Devlieghere F, Smet K, Standaert AR, Geeraerd AH, Van Impe JF, Debevere J

(2005) Int J Food Microbiol 100:41-53

[24] Francois K, Devlieghere F, Standaert AR, Geeraerd AH, Van Impe JF, Debevere J (2006) Int J Food Microbiol 108:326-335

[25] Ponciano JM, Vandecasteele FPJ, Hess TF, Forney LJ, Crawford RL, Joyce P (2005) Appl Environ Microbiol 71:2355-2364.

[26] Spagnolo B, Valenti D, Fiasconaro A (2004) Math Bios and Eng 1:185-211.

[27] Valenti D, Fiasconaro A, Spagnolo B (2004) Physica A 331:477-486.

[28] Giuffrida A, Ziino G, Rago F, Giarratana F, Taviano A, Panebianco A (2005)In Proceeding of INTRADFOOD 2005, Innovation in Traditional Food, Valencia Spain, pp 537-540.

[29] Le Marc Y, Pin C, Baranyi J (2005) Int J Food Microbiol 100:3-12.

[30] Leroy F, De Vuyst L (1999) Appl Environ Microbiol 65:974-981.

[31] Leroy F, Degeest B, De Vuyst L (2002) Int J Food Microbiol 73:251-259.

[32] Wijtzes T, Rombouts FM, Kant-Muermans MLT, van't Riet K, Zwietering MH (2001) Int J Food Microbiol 63:57-64.

[33] Gardiner C W (2004) Handbook of Stochastic Methods. Springer-Verlag, Berlin.

[34] Campanini M, Pedrazzoni I, Barbuti S, Baldini P (1993) Int J Food Microbiol 20:169-175.

[35] Trussel M, Jemmi T (1989) Fleischwirtsch 69:1586-1592.

[36] Villani F, Sannino F, Moschetti G, Mauriello G, Pepe O, Amodio-Cocchieri R, Coppola S (1997) Food Microbiol 14, 555-566.

[37] Nissen H, Holck A (1998) Food Microbiol 15:273-279.

[38] Tyopponen S, Markkula E, Petaja M, Suihko T, Mattila-Sandholm T (2003) Food Control 14:181-185.

[39] Scott V, Swanson KMJ, Freier TA, Payton Pruett JR, Sveum W, Hall P, Smoot LA, Brown D (2005) Food Prot. Trends 11: 818-825.

[40] Besse NG, Audinet N, Barre L, Cauquil A, Cornu M, Colin P (2006) Int J Food Microbiol 110:43-51.

[41] Leroy F, Kristoff L, De Vuyst L (2005) Appl Environ Microbiol 71: 7567-7570.

[42] Giraffa G (2004) FEMS Microbiol Rev 28:251-260.

[43] Pouillot R, Albert I, Cornu M, Denis JB (2003) Int J Food Microbiol 81:87-104.

[44] Gallagher R, Appenzeller T (1999) Science 284, 79 (one page); see all the issue "Complex Systems" (1999) Science 284:79-107.

[45] Zimmer C (1999) Science 284:83-86.

[46] Bjørnstad ON, Grenfell BT (2001) Science 293:638-643.

[47] Benzi R, Sutera A, Vulpiani A (1981) J Phys A: Math Gen 14:L453-L457.

[48] Benzi R, Parisi G, Sutera A, Vulpiani A (1982) Tellus 34:10-16.

[49] Jung P, Hnggi P (1989) Europhys Lett 8:505-510.

[50] Jung P, Hnggi P (1991) Phys Rev A 44:8032-8042.

[51] Mantegna RN, Spagnolo B (1994) Phys Rev E 49:R1792- R1795.

[52] Lanzara E, Mantegna RN, Spagnolo B, Zangara R (1997) Am J Phys 65:341-349.

[53] Gammaitoni L, Hnggi P, Jung P Marchesoni F (1998) Rev Mod Phys 70:223-287.

[54] Alley R B, Anandakrishnan S, Jung P (2001) Paleoceanogr 16:190-198.

[55] Mantegna RN, Spagnolo B, Trapanese M (2001) Phys Rev E 63:1-8.

[56] Mantegna RN, Spagnolo B (1996) Phys Rev Lett 76:563-566.

[57] Mantegna RN, Spagnolo B (1998) Int J Bifurcation and Chaos 8:783-790.

[58] Agudov NV, Spagnolo B (2001) Phys Rev E 64:1-4.

[59] Agudov N V, Dubkov A A, Spagnolo B (2003) Physica A 325:144-151.

[60] Fiasconaro A, Valenti D, Spagnolo B (2003) Phys A 325:136-143.

[61] Spagnolo B, La Barbera A (2002) Physica A 315:114-124.

[62] Freund JA, Pschel T (2000) Lecture Notes in Physics 557 Berlin: Springer.

[63] Valenti D, Tranchina L, Brai M, Caruso A, Cosentino C, Spagnolo B (2008) Ecol Model 213:449-462

[64] Ciuchi S, de Pasquale F, Spagnolo B (1996) Phys. Rev E 54:706-716

[65] Ratkowsky DA (2002) Int J Food Microbiol 73:119-125 65

[66] Valero A, Carrasco E, Prez-Rodriguez F, Garca-Gimeno RM, Zurera G (2006) Eur Food Res Technol 224: 91-100 doi: 10.1007/s00217-006-0293-1.